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14. ABSTRACT Molecular targets to facilitate early detection and preventative therapy for women at high risk for breast cancer have not been characterized. Two recently characterized intracellular enzymes -- human telomerase reverse transcriptase (hTERT) and the cytochrome P450 isoform 1B1 (CYP1B1), each overexpressed in >90% of invasive breast cancers but rarely found in normal tissue -- may fill this gap. Such targets, if found at the earliest time of malignant transformation, may be ideally suited not only for early detection but also cancer prevention by vaccination. A growing clinical experience in advanced cancer patients has underscored the safety and feasibility of vaccination strategies. The universal expression of hTERT and CYP1B1 provide an opportunity for both early detection and cancer vaccination. We hypothesize that the candidate universal tumor antigens hTERT and CYP1B1 can be detected in ductal lavage specimens, specific for the presence of carcinoma. We further hypothesize that immunologic responses can be elicited in advanced breast cancer patients using vaccines incorporating hTERT, providing a safety and feasibility platform for ultimately vaccinating women at high risk for breast cancer.					
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Progress Report
Department of Defense Physician-Scientist Award
Universal Breast Cancer Antigens as Targets Linking Early Detection and Therapeutic Vaccination

Susan M. Domchek, MD
September 9, 2005

SPECIFIC AIMS OF THE PROJECT

- 1. Evaluation of molecular markers in ductal lavage fluid from BRCA1 and BRCA2 mutation carriers**
- 2. Determine the safety and feasibility of vaccinating advanced breast cancer patients with hTERT peptide, assessing the generation of hTERT-specific immunity. Explore the role of intravenous cyclophosphamide prior to hTERT vaccination in boosting vaccine response by depleting regulatory T cells.**

A. INTRODUCTION

This grant supports studies to understand the potential of universal tumor antigens for cancer immunotherapy, with a particular focus on the characterization of the human telomerase reverse transcriptase (hTERT) as tumor antigen. Telomerase is expressed by >90% of all human breast cancers but absent in most normal cells. Telomerase function has been directly linked to oncogenesis and its inhibition in telomerase-positive human tumors leads to growth arrest.

Following a series of published in vitro preclinical experiments, we are now testing the hypothesis of telomerase as a tumor rejection antigen in vivo in humans. This year we completed enrollment in the dose escalation portion of our hTERT peptide vaccine trial. Immunologic responses were assessed and the optimal dose level was chosen and an additional four patients were treated at that dose. Furthermore, we have initiated work examining the role of intravenous cyclophosphamide prior to hTERT vaccination in an attempt to boost vaccine response by depleting regulatory T cells. Data thus far from our past and current trials suggest that telomerase peptide vaccination is biologically active and leads to in vivo immune recognition of carcinoma by effector lymphocytes and tumor necrosis.

We remain hopeful that ductal lavage will be a mechanism by which to assess presence of tumor-associated antigens and therefore the potential for preventative vaccination. Evidence of immunologic response to hTERT based vaccines in advanced cancer patients, as well as demonstration of safety and feasibility, would justify the pursuit of preventative vaccination in high risk individuals, particularly those in whom such antigens are expressed.

B. BODY

Aim 1: Evaluation of molecular markers in ductal lavage fluid from BRCA1 and BRCA2 mutation carriers

Goals at year two according to revised statement of work: Prepare protocol for ductal lavage in BRCA1 and BRCA2 carriers. We are now collaborating with Dr. Gillian Mitchell's group in Australia examining molecular markers present in ductal lavage specimens from BRCA1 and BRCA2 mutation carriers. The protocol has been prepared and will be submitted within the month. Once submitted, we will forward a copy of the protocol to the Department of Defense grants officer. In April of 2005, Dr. Domchek became the director of the Cancer Risk Evaluation Program at the Abramson Cancer Center of the University of Pennsylvania. In this position, she has overall responsibility for the care and follow-up of BRCA1 and BRCA2 mutation carriers at the university. Yearly follow-up is obtained on over 600 BRCA1 and BRCA2 mutation carriers at the University of Pennsylvania and therefore the patient population from which to accrue to this protocol is large.

Aim 2: Determine the safety and feasibility of vaccinating advanced breast cancer patients with hTERT peptide, assessing the generation of hTERT-specific immunity. Explore the role of intravenous cyclophosphamide prior to hTERT vaccination in boosting vaccine response by depleting regulatory T cells.

Goals at year two according to revised statement of work: Complete peptide only trial, analyze data and prepare for publication. In the dose escalation portion of our phase I clinical trial, 16 HLA-A2+ women with metastatic breast cancer have been vaccinated subcutaneously with 10 ug (n=6), 100 ug (n=5), or 1000 ug (n=5) of hTERT I540 peptide emulsified in Montanide adjuvant and administered with GM-CSF for up to 8 vaccinations per UPCC Protocol 11102 (Domchek PI) and under the auspices of BB-IND 10675 (Vonderheide sponsor). No dose limiting toxicities have been observed, including no bone marrow toxicity. Grade 1 and 2 injection site reactions have been observed in most patients. In the majority of patients, at all dose levels, we have observed a syndrome of tumor pain or tumor-site itchiness after vaccination. Patients who completed all 8 vaccinations were eligible to continue receiving monthly vaccinations until the time of disease progression. One of 5 evaluable patients at dose level one completed all 8 vaccinations and has stable disease (14 months before progression) and four patients had progressive disease after completing 3, 6, 4, and 4 vaccinations. At the second dose level, 5 patients are evaluable, and one has had a minor response (chest wall tumor regression, s/p 7 vaccinations and 5+ months before progression), 2 patients have stable disease (s/p all 8 vaccinations and stable at 9 months before progression; s/p 8 vaccinations and stable at 19+ months and still receiving vaccines), and two patients had progressive disease after completing 6 vaccinations each. At the third dose level four patients had progressive disease after completing 4, 4, 4, and 5 vaccines. A fifth patient has stable disease (s/p all 8 vaccinations and stable at 13+ months and still receiving vaccines). As will be discussed below, immunoassessment was performed on all three dose levels. Dose level two (100 ug) was felt to be the most immunogenic and an additional four patients have been enrolled on this dose. Staging and immunologic assessments of these patients are pending. We have also enrolled our first patient on the cyclophosphamide portion of the protocol and she has received her first vaccine.

Screening immunoassessment currently includes tetramer analysis of freshly isolated PBMC and tetramer analysis of PBMC stimulated for one week with hTERT peptide vs. control peptide. In two patients at the 10 ug dose level, 4 of 5 patients at the 100 ug level and 2 of 5 patients at the 1000 ug level, hTERT-specific CD8+ T cells were observed after vaccination (0.10% to 2.0%

hTERT tetramer+ CD8+ T cells among freshly isolated or in vitro stimulated CD8+ peripheral blood cells after vaccination compared to $\leq 0.05\%$ before). These cells could be specifically expanded by in vitro stimulation and have been now shown to kill tumor cells in vitro. All four patients who completed all eight vaccines and then continued on to further vaccination had evidence of an immune response. Two other patients with immunologic responses had evidence of hTERT specific tumor infiltrating lymphocytes on tumor biopsy. Therefore 6 of 8 patients with immunologic responses had either pathologic evidence of vaccine effect or possibly had clinical benefit with a long period of stable disease.

Five patients have undergone tumor biopsy on study, and flow cytometric and histopathologic analyses have been performed. In 3 patients, hTERT-specific CD8+ tumor infiltrating lymphocytes were observed by tetramer analysis (between 5%-12% tetramer+ cells among CD8+ T cells compared to a negative tetramer control stain of $\leq 0.06\%$ CD8+ T cells). In two patients, TILs were associated with marked tumor necrosis involving 50%-80% of the biopsy specimens. In one of these patients, serial tumor biopsies revealed that the induction of TILs preceded tumor necrosis by 5 months.

Therefore, in the past year, our data provide in vivo evidence that I540 telomerase peptide vaccination leads to in vivo immune recognition of carcinoma by effector lymphocytes and tumor necrosis. We have expanded the cohort at the 100 ug dose level to gain additional information about immunogenicity and toxicity. Finally, we have begun to examine the role of cyclophosphamide as an immune modulating agent prior to the administration of hTERT vaccine, with the first patient being enrolled on this portion of the protocol this month.

Plans for Year Two

Plans for the next year of support include work on SPECIFIC AIMS ONE and TWO.

For AIM ONE, we will examine molecular markers in ductal lavage fluid of BRCA1 and BRCA2 mutation carriers.

For AIM TWO: Publication of the hTERT peptide vaccine trial. Continue enrollment on cyclophosphamide portion of the study.

KEY RESEARCH ACCOMPLISHMENTS

1. Completion of dose escalation portion of protocol UPCC 11102
2. Completion of immunologic assessments for patients undergoing dose escalation portion of UPCC 11102
3. Selected for oral presentation at the American Society of Clinical Oncology annual meeting in 2005 entitled: "Telomerase vaccination of metastatic breast cancer patients induces antigen-specific tumor infiltrating lymphocytes and tumor necrosis"
4. Selected for oral presentation at the Department of Defense "Era of Hope" meeting in 2005 entitled: "Telomerase vaccination of metastatic breast cancer patients induces antigen-specific tumor infiltrating lymphocytes and tumor necrosis"
5. IND and IRB approval for the use of intravenous cyclophosphamide as an immune modulator prior to hTERT vaccination

REPORTABLE OUTCOMES:

A. Publications During This Funding Period (2004-2005)

1. Halbert C, Brewster K, Collier A, Smith C, Kessler L, Weathers B, Stopfer J, **Domchek S**, and Wiley EP. Recruiting African American Women to Participate in Hereditary Breast Cancer Research. *Journal of Clinical Oncology*, in press.
2. Danet G, Leibowitz M, **Domchek SM**, Vonderheide RH. Bone marrow from patients receiving telomerase vaccination reconstitutes bone marrow in a NOD-SCID mouse model. *Experimental Hematology*, in press.
3. Rebbeck TR, Friebe R, Wagner R, Lynch HT, Garber JE, Daly MB, Isaacs C, Olopade O, Neuhausen SL, Van't Veer L, Eeles R, Evans F, Tomlinson G, Matloff E, Narod SA, Eisen A, **Domchek S**, Armstrong K, Weber BL. Effect of short term hormone replacement therapy on breast cancer risk reduction after bilateral prophylactic oophorectomy in *BRCA1* and *BRCA2* mutation carriers. *Journal of Clinical Oncology*. 2005; in press.
4. Peters N, **Domchek SM**, Rose A, Polis R, Stopfer J, Armstrong K. Knowledge, attitudes and utilization of BRCA1/2 testing among women with early onset breast cancer. *Genetic Testing*. 2005; 9: 48-53.
5. **Domchek SM**, Merillat S, Tigges J, Tweed AJ, Weinart M, Stopfer J, Weber BL. Sex ratio skewing of offspring in families with hereditary susceptibility to breast cancer. *J Med Genet*. 2005; 42: 511-13.
6. Schwartz J, **Domchek SM**, Hwang W, Fox K. Evaluation of anemia, neutropenia, and skin toxicities in standard or dose-dense doxorubicin/cyclophosphamide (AC)-paclitaxel or docetaxel adjuvant chemotherapy in breast cancer. *Annals of Oncology*. 2005; 16: 247-52..
7. Gurmankin AD, **Domchek SM**, Stopfer J, Fels C, Armstrong K. Patients' resistance to risk information in genetic counseling for BRCA1/2. *Archives of Int Med*. 2005, 165: 523-9.
8. Huang J, **Domchek SM**, Brose MS, Rebbeck TR, Nathanson KL, Weber BL. Germline *CHEK2**1100delC mutations in breast cancer patients with multiple primary cancers. *Am J Hum Gen*. 2004; 41:e120.
9. Domchek SM and Weber BL. Guidelines for genetic counseling, testing and referral in North America. Chapter in *Risk Assessment and Management in Cancer Genetics*. Eds. Laloo F, Kerr B, Friedman JM, Evans DGR. 2005.
10. Schachter M and Domchek SM. Update in chemoprevention in BRCA1 and BRCA2 mutation carriers. *Breast Cancer Online*. 2005.

B. Abstracts

1. Domchek SM, Fox KR, Recio A, Schuchter LM, Davidson R, DeMichele A, Feldman MD, Vonderheide RH. "Telomerase vaccination of metastatic breast cancer patients induces antigen-specific tumor infiltrating lymphocytes and tumor necrosis" Oral Presentaion, ASCO, Orlando FL, 2005
2. Domchek SM, Fox KR, Recio A, Schuchter LM, Davidson R, DeMichele A, Feldman MD, Vonderheide RH. "Telomerase vaccination of metastatic breast cancer patients induces antigen-specific tumor infiltrating lymphocytes and tumor necrosis" Oral Presentaion, Department of Defense Era of Hope Meeting, Philadelphia, 2005

C. Funding

Dr. Domchek is a co-investigator on a RO1 which was funded this year. This grant permits the evaluation of I540 peptide vaccination in combination with anti-CD25 mAb in a further attempt to boost immune response by depleting regulatory T cells. The principle investigator on the grant is Dr. Robert Vonderheide, and the grant number is Ro1 CA111377-01A1. Dr. Domchek will be the principle investigator on the clinical trial which will be part of the grant.

CONCLUSIONS

Data thus far from our current trial suggest that telomerase peptide vaccination is biologically active and leads to in vivo immune recognition of carcinoma by effector lymphocytes and tumor necrosis. This has great potential for biological therapy of breast cancer and required further exploration. If hTERT expression can be found in women at high risk for breast cancer, this may represent a marker to be used to target candidates for vaccination in the future.

REFERENCES (See "Publications" in "Reportable Outcomes")

APPENDICES

1. Domchek CV
2. Revised Statement of Work (previously submitted to grants officer)
3. Updated clinical protocol

UNIVERSITY OF PENNSYLVANIA - SCHOOL OF MEDICINE
Curriculum Vitae

June, 2005

Susan M. Domchek, M.D.

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University of Pennsylvania Cancer Center
3400 Spruce Street
Philadelphia, PA 19104
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FAX: 215-615-3349

Education

9/86-6/90	B.A.	Dartmouth College, Hanover, NH (Engineering Sciences)
9/93-6/94		Oxford University, England (English Literature)
8/90-6/95	M.D.	Harvard Medical School

Postgraduate Training and Fellowship Appointments

7/95-6/96	Intern, Internal Medicine, Massachusetts General Hospital
7/96-6/98	Resident, Internal Medicine, Massachusetts General Hospital
7/98-7/01	Clinical Fellow in Hematology and Oncology, Dana-Farber Cancer Institute
1/00-12/00	Chief Medical Resident, Massachusetts General Hospital

Faculty Appointments

1/00-10/01	Instructor in Medicine, Harvard University
11/01-	Assistant Professor in Medicine, University of Pennsylvania

Hospital and Administrative Appointments

1/00-6/01	Assistant in Medicine, Massachusetts General Hospital
7/01-10/01	Assistant in Medicine, Dana-Farber Cancer Institute
4/05-	Director of the Cancer Risk Evaluation Program, Abramson Cancer Center, University of Pennsylvania

Specialty Certification

1998	American Board of Internal Medicine
2001	American Board of Internal Medicine: Medical Oncology

Licensure

1998	Massachusetts
2001	Pennsylvania

Awards, Honors and Membership in Honorary Societies

1989	Choate Scholar, Dartmouth College
1989	Phi Beta Kappa, Dartmouth College
1990	<i>Summa cum laude</i> , Dartmouth College
1993	Marshall Scholar, Oxford University
1995	<i>Magna cum laude</i> , Harvard Medical School
2000	Chief Medical Resident, Massachusetts General Hospital
2001	Landenberger Scholar, University of Pennsylvania
2002	Ann B. Young Assistant Professor in Cancer Research, University of Pennsylvania

2003 Department of Defense, Physician-Scientist Award
 2003 Tracey Starr Award

Memberships in Professional and Scientific Societies

1999- American Society of Clinical Oncology

Editorial Positions

2000- Ad Hoc reviewer, *Cancer*
 2001- Ad Hoc reviewer, *Journal of Clinical Oncology*
 2002- Ad Hoc reviewer, *New England Journal of Medicine*
 2002- Ad Hoc reviewer, *Journal of Medical Genetics*
 2002- Ad Hoc reviewer, *Clinical Cancer Research*
 2003- Ad Hoc reviewer, *Journal of General Internal Medicine*

Academic Committees at Massachusetts General Hospital

1999-2000 Internship Selection Committee, Internal Medicine Residency
 2000 Internal Review of Pediatrics Program
 2000 Teaching and Training Council, Internal Medicine Resident
 2000 Curriculum Committee, Internal Medicine Residency
 2000 Training Program Council, Internal Medicine Residency

Academic Committees at University of Pennsylvania

2003 Educational Taskforce for Department of Medicine Strategic Planning Initiative

Major Teaching Responsibilities at Harvard University

2000 Chief Resident in Medicine, Massachusetts General Hospital

- Planned and led daily residents' reports
- Provided case discussion at multiple residents' reports
- Planned and supervised daily lecture series for the medical housestaff
- Planned and ran weekly Morbidity & Mortality conferences
- Provided case discussion at multiple Morbidity & Mortality conferences
- Served as ward attending for two months and supervised team of residents, interns and medical students
- Taught at case conferences for Harvard Medical students
- Served as medical consult attending for one month and supervised team of residents
- Directed and organized the entire medical residency program which included 130 interns and residents

8/00 "Chemotherapy concepts for house officers",
 Massachusetts General Hospital Medical Housestaff lecture series, Boston, MA

2/01 "Breast cancer", Massachusetts General Hospital Medical Housestaff lecture series, Boston, MA

3/01 Harvard Medical Student Subinternship teaching series, monthly presentation, Boston, MA

9/01 "Breast cancer", Massachusetts General Hospital Medical Housestaff lecture series. Boston, MA

Major Teaching Responsibilities at University of Pennsylvania

11/01- Assistant Professor of Medicine, University of Pennsylvania

- Serve as inpatient attending for four weeks a year, supervising team of fellows, residents, interns and medical students
- Serve as inpatient oncology consult four weeks a year, supervising oncology fellows

- Preceptor to medical students and residents in outpatient clinic
- Preceptor to residents in the Women's Health Elective
- 7/02 "Cancer screening trials", Educational series for medical oncology fellows
- 5/03 "Breast cancer genetics", Medical student, endocrinology course
- 5/03 "Tamoxifen decision-making", Medical student decision making course
- 5/04 "Breast cancer genetics", Medical student women's health course
- 6/04 "Adjuvant therapy in breast cancer", University of Pennsylvania radiation oncology residents, June 1 2004
- 7/04 "Cancer screening trials", Educational series for medical oncology fellows
- 2/05- Doctoring I – longitudinal course with medical students
- 3/05 "Hereditary breast and ovarian cancer", OB/GYN fellowship lecture series
- 5/05 "Breast cancer genetics", Medical student endocrine course
- 7/05 "Cancer screening trials", Educational series for medical oncology fellows

Lectures by Invitation

National

- 8/98 Fellow Conference on Breast Cancer, M.D. Anderson Cancer Center "Predictors of skeletal complications in metastatic breast cancer", Houston, TX
- 11/98 New England Cancer Society Annual Meeting, "Predictors of skeletal complications in metastatic breast cancer", Worcester, MA.
- 1/03 "Management of BRCA1 and BRCA2 mutation carriers", San Antonio Update, Baylor College of Medicine, Washington D.C.
- 5/04 "Update in adjuvant therapy for breast cancer", Teich Lecture, Beth-Israel Medical Center, New York
- 5/04 "Risk models in clinical practice", National Cancer Institute Risk Modeling Meeting, Washington, DC
- 1/05 "Telomerase immunotherapy of breast cancer", Breast Cancer Think Tank 15, Curacao, Dutch Antilles
- 4/05 "Genetic Susceptibility and Breast Cancer", New Strategies in Breast Cancer 2005, CME sponsored by CBCE and led by Drs. George Sledge and Dr. Dennis Slamon
- 4/05 "Hereditary Breast and Ovarian Cancer Syndromes" at the 1st Annual Women's Health Summit sponsored by the Cleveland Clinic Foundation Women's Health Center, Cleveland, OH
- 4/05 "Genetics and Women at High Risk for Breast Cancer" at the 1st Annual Women's Health Summit sponsored by the Cleveland Clinic Foundation Women's Health Center, Cleveland, OH
- 5/05 "How to write a clinical trial", Educational session at American Society of Clinical Oncology, Orlando, FL
- 5/05 "Telomerase vaccination of metastatic breast cancer patients induces antigen-specific tumor infiltrating lymphocytes and tumor necrosis", Selected Oral Abstract Presentation, American Society of Clinical Oncology, Orlando, FL
- 6/05 "Telomerase vaccination of metastatic breast cancer patients induces antigen-specific tumor infiltrating lymphocytes and tumor necrosis," Selected Oral Abstract Presentation, Department of Defense Era of Hope Meeting, Philadelphia, PA

Regional

- 5/01 "Breast cancer, risk and prevention", Newton-Wellesley Hospital, Newton, MA
- 6/01 "Hormonal replacement therapy and the risk of breast cancer", Living Well series, Dana-Farber Cancer Institute, Boston, MA

7/01 oncology	"Hormonal therapies in breast cancer", Educational series for radiation residents, Dana-Farber Cancer Institute, Boston, MA
9/02	"Ductal lavage for the detection of breast cancer: how it works and what's the evidence?", Changing Concepts in Breast Cancer 2002 Conference, University of Pennsylvania, Philadelphia, PA
10/02	"Breast cancer: risk, screening, prevention and management", Moravian College, Bethlehem, PA
10/02	"Breast cancer genetics: who to test and how to manage", Moravian College, Bethlehem, PA
10/02 PA	"What is a clinical trial?", Pennsylvania Breast Cancer Coalition, Harrisburg, PA
10/02	"Ductal lavage", Life After Breast Cancer, University of Pennsylvania, Philadelphia, PA
11/02	"Hormone replacement therapy and breast cancer risk", FOCUS panel discussion, University of Pennsylvania, Philadelphia, PA
6/03	"Update on breast cancer susceptibility genes". Medical Grand Rounds, Chester County Hospital, West Chester, PA.
8/03	"Breast, ovarian and colon cancer genetics." Medical Grand Rounds. Pocono Medical Center, East Stroudsburg, PA
9/03	"Breast cancer genetics", Life After Breast Cancer, University of Pennsylvania, Philadelphia, PA
9/03	"Breast cancer genetics: How to test and how to manage". Medical Grand Rounds. Lancaster General Hospital, Lancaster, PA.
1/04	"Breast cancer genetics". Medical Grand Rounds, St. Joseph's Hospital, Reading, PA
6/04	"ASCO update: Breast cancer prevention, detection and genetics", University of Pennsylvania CME course, Philadelphia PA
9/04	"Breast cancer genetics", Life After Breast Cancer Conference, University of Pennsylvania, Philadelphia, PA
10/04	"Breast cancer overview", Moravian College, Bethlehem, PA
4/05	"An Update on Breast Cancer Genetics", Changing Concepts in Breast Cancer, University of Pennsylvania CME Symposium, Philadelphia, PA

Organizing Roles in Scientific Meetings

2004-	American Society of Clinical Oncology, Education Committee, Tumor Biology and Genetics
2004	Chairman, "Risk Modifiers in Hereditary Cancer Syndromes" American Society of Clinical Oncology Annual Meeting, Orlando Florida
2005-	American Society of Clinical Oncology, Track Leader, Cancer Genetics Education Committee

Bibliography

Research publications, peer reviewed

1. **Domchek SM**, Greshock J, Tweed, Jessica Tigges AJ, Blackwood-Chirchir MA, Yang HP, Nathanson K, Stratton M, Easton D, Stopfer JE, Olopade OI, Rebbeck TR, and Weber BL. A Web-Based Prediction Model for *BRCA1* and *BRCA2* Mutations. *Journal of Clinical Oncology*, submitted.
2. Lehman CD, Isaacs C, Schnall MD, Pisano E, Ascher SM, Weatherall PT, Bluemke DA, Bowen D, Marcom PK, Armstrong DK, **Domchek S**, Tomlinson GE, Skates SK, and Gatsonis C. Cancer Yield

- of Mammography, MRI, and Ultrasound in High Risk Women Enrolled in a Prospective Multi-institution Breast Cancer Screening Trial. *Journal of Clinical Oncology*, submitted.
3. Halbert C, Brewster K, Collier A, Smith C, Kessler L, Weathers B, Stopfer J, **Domchek S**, and Wiley EP. Recruiting African American Women to Participate in Hereditary Breast Cancer Research. *Journal of Clinical Oncology*, in press.
 4. Danet G, Leibowitz M, **Domchek SM**, Vonderheide RH. Bone marrow from patients receiving telomerase vaccination reconstitutes bone marrow in a NOD-SCID mouse model. *Experimental Hematology*, in press.
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 8. Schwartz J, **Domchek SM**, Hwang W, Fox K. Evaluation of anemia, neutropenia, and skin toxicities in standard or dose-dense doxorubicin/cyclophosphamide (AC)-paclitaxel or docetaxel adjuvant chemotherapy in breast cancer. *Annals of Oncology*. 2005; 16: 247-52..
 9. Gurmankin AD, **Domchek SM**, Stopfer J, Fels C, Armstrong K. Patients' resistance to risk information in genetic counseling for *BRCA1/2*. *Archives of Int Med*. 2005, 165: 523-9.
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 13. Stein S, DeMichele A, **Domchek S**, Fox K. Gemcitabine and trastuzumab combinations for patients with metastatic breast cancer overexpressing HER2/neu. *Clinical Breast Cancer*. 2004; 4:S117-120.
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Editorials, Reviews, Chapters

1. Domchek SM and Garber JE. Genetic Testing for Breast Cancer. *Principles & Practice of Oncology: Updates*. 2001; 15: 1-15.
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Revised Statement of Work
Department of Defense Physician-Scientist Award
Universal Breast Cancer Antigens as Targets Linking Early Detection and Therapeutic
Vaccination
DAMD17-03-1-0619

Susan M. Domchek, MD

AIM 1:

Task: Evaluation of molecular markers in ductal lavage fluid from BRCA1 and BRCA2 mutation carriers

Timeline:

1. Attempt to enroll patients with BIRADS category 4 mammograms on ductal lavage study (0-12 months) limited by poor accrual
2. Establish relationship with Dr. Gillian Mitchell at the Peter MacCollum Cancer Center exploring role of ductal lavage in BRCA1/2 mutation carriers (12-24 months)
3. Prepare protocol examining molecular markers in ductal lavage fluid in BRCA1/2 carriers for submission to IRB and obtain approval (20-26 months)
4. Enroll patients onto and obtain data for ductal lavage protocol (26-36 months)

AIM 2:

Task: Determine the safety and feasibility of vaccinating advanced breast cancer patients with hTERT peptide, assessing the generation of hTERT-specific immunity. Explore the role of intravenous cyclophosphamide prior to hTERT vaccination in boosting vaccine response by depleting regulatory T cells.

1. Obtain FDA approval of the Investigational New Drug (IND) application for the manufacture of hTERT peptide vaccine (0-3 months)
2. Obtain final University of Pennsylvania Cancer Center IRB approval for Protocol #11102 "Phase I Study of Telomerase Peptide Vaccination for Patients with Advanced Breast Cancer" (0-3 months)
3. Recruit and enroll subjects (0-24 months)
4. Determine safety and clinical responses (3-24 months)
5. Assess immunologic responses (3-24 months)
6. Analyze data and prepare manuscript for publication (24-30 months)
7. Obtain final University of Pennsylvania Cancer Center IRB approval for amendment to UPCC #11102 exploring the role of intravenous cyclophosphamide prior to hTERT vaccination in boosting vaccine response by depleting regulatory T cells (18-24 months)
8. Recruit and enroll subjects on cyclophosphamide arm of study (24-36 months)
9. Determine safety and clinical responses (30-36 months)

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**Phase I Study of Telomerase Peptide Vaccination for
Patients with Advanced Breast Cancer**

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1 BACKGROUND AND SIGNIFICANCE

1.0 Introduction

Studies in both human and animal systems provide compelling evidence that the immune system can be manipulated to specifically recognize human tumor cells and kill them (1). Most attention has focused on the CD8+ cytotoxic T lymphocyte (CTL) as the primary effector cell of antigen-specific anti-tumor immunity especially in light of recent findings that tumor-derived proteins and peptides function as tumor-associated antigens (TAA) and targets for CTL (2, 3). Clinical efforts, however, have been limited in part because most tumor antigens are restricted in expression to one or a few tumor types and to a fraction of patients with these types of tumors (1). This protocol makes use of the novel TAA telomerase that can potentially trigger T cell responses against a broad range of tumor types, including breast cancers. In a recent report, a peptide derived from the telomerase reverse transcriptase (also known as hTERT) was identified as a nearly universal TAA recognized by CTL (4). Its expression in more than 85% of human cancers but few normal tissues (5) makes it the most universal tumor antigen yet described (6).

In a recently completed vaccination trial using autologous monocyte-derived dendritic cells expressing hTERT peptide, there was minimal toxicity in 7 patients treated despite evidence that hTERT immunity was successfully induced after vaccination. In addition, the two breast cancer patients who participated in this study mounted an anti-hTERT immunological response

following vaccination, and one patient had evidence for a mixed clinical response (7).

In the current protocol, which builds on our previous clinical experience with hTERT vaccination, patients with advanced breast cancers will be treated with hTERT peptide emulsified in the adjuvant Montanide ISA 51 and given together with the cytokine granulocyte-macrophage colony stimulating factor (GM-CSF). The vaccine will be given subcutaneously 8 times over 27 weeks. The primary endpoints will be (i) safety and toxicity, and (ii) the assessment of the generation of hTERT peptide-specific CTL immune response.

1.1 Clinical progress in tumor immunity and peptide vaccination

In the last several years, scientific advances in T cell biology and tumor immunology have greatly facilitated efforts to develop novel T-cell based therapy for the treatment of cancer (1). In tumor-specific vaccination strategies, patients are immunized against tumor-specific or tumor-associated targets in order to activate specific immunity against cancer, generally in a therapeutic setting. Most vaccination approaches have been shown to be highly feasible. Numerous trials using a variety of antigen-delivery systems have been safely completed, and many more are currently underway. Early reports in melanoma and hematologic malignancies suggest that antigen-specific vaccination is safe, feasible, and potentially effective (8, 9). More recently, this approach has been applied to carcinomas, including tumors of the pancreas, breast, ovary, colon, and cervix. These trials also show very low levels of toxicity, with some early suggestion of clinical efficacy (10, 11).

One common clinical approach for antigen-specific tumor vaccination has been the experimental use of peptides derived from tumor antigens in the immunizing inoculums. The approach of peptide vaccination has recently been extensively reviewed (12, 13). Table 1 highlights several such trials as an illustration of the feasibility and safety of this approach. The general features of these protocols include multiple subcutaneous or intradermal injections of peptide with adjuvant and/or cytokines given over a course of weeks. The number of patients who have completed these trials is large, and clinical experience with peptide vaccines had demonstrated that they appear to be very well tolerated. Most side effects have been minor, and are largely related to inflammation at the injection site. Many clinical trials of peptide vaccination in cancer patients have elicited measurable cellular and/or humor immune responses to the vaccine. Occasional objective tumor responses have also been observed, but there is much still to be learned about which TAA to target, and how to induce a clinically meaningful and long-lasting immune response.

A variety of peptide doses up to 1000 μg per injection have been used, but the optimal dose of TAA peptide for vaccination has not been defined. It is also not known how the use of adjuvant, cytokines, or both with peptide vaccination alters the pattern of dose response in treated patients. Thus, in this study, we incorporate the evaluation of three peptide dose levels spanning the range of peptide doses previously tested, from 10 μg to 1000 μg .

Table 1. Clinical Trials of Peptide Vaccination

Disease	Antigen	Adjuvant	N	# Imm. Resp.	Toxicity >II	Ref
Advanced solid tumors	NY-ESO1	GM-CSF 75µg x 4	12	5	0	(14)
Advanced solid Tumors	Ras	GM-CSF 40µg x 1	48	21	0	(11)
Advanced solid tumors	Ras	Detox	15	3	0	(15)
Breast and Ovarian	Her2/neu	GM-CSF 125µg x 1 Helper epitopes	18	14	0	(16)
Breast and Ovarian	Her2/neu	GM-CSF 125µg x 1	8	8	0	(10)
Cervical Ca	HPV E7	Montanide PADRE	18	10	0	(17)
CML	Bcr-abl	QS-21	12	5	0	(18)
Colorectal	CEA protein	+/-GM-CSF x 4	9	9	0	(19)
Colorectal	SART3	Montanide	12	7	0	(20)
Melanoma	gp100 gp100(m) gp100(m)	Montanide Montanide Montanide plus IL2	9 11 19	23	0	(8)
Melanoma	gp100	Tetanus QS-21 or Montanide	22	3	2	(21)
Melanoma	gp100 Tyrosinase	Montanide +/- IL12	48	37	8	(22)
Melanoma	MAGE1	None	25	0	0	(23)
Melanoma	MART 1 gp100 Tyrosinase Influenza	None	6	5	0	(24)
Melanoma	MART1 Tyrosinase gp100 Influenza	GM-CSF 75µg x 5	3	3	0	(25)
Melanoma	MART1	Montanide	23	12	0	(26)
Melanoma	MART1	Montanide	25	11	2	(27)
Melanoma	Ras	GM-CSF 40µg x 1	10	8	0	(28)
Melanoma	Tyrosinase	QS-21	9	2	0	(29)
TOTAL			362	186	12	

1.2 Identification of the telomerase reverse transcriptase hTERT as a nearly universal tumor associated antigen

Increasing data, which has recently been extensively reviewed (6), suggests that hTERT can serve as a target for widely applicable immunotherapy against cancer. Peptides derived from hTERT are naturally processed by tumor cells, presented in an MHC class I-restricted fashion, and serve as a target for antigen-specific CTL (4, 30). Telomerase activity is found in >85% of all human cancers but few normal tissues (5, 31), and maintains telomeric ends of linear chromosomes and protects them from degradation and end-to-end fusion (32). As the rate-limiting component of telomerase function, hTERT expression correlates best with telomerase activity (33). Most human cells do not have telomerase activity or hTERT expression (33, 34). In contrast, the great majority of human tumors exhibit strong telomerase activity, express hTERT, and maintain the length of their telomeres (33, 34). Expression of telomerase has been directly linked to the development of human cancers (35), and its inhibition in telomerase-positive tumors results in tumor death *in vitro* without the outgrowth of telomerase-negative tumor cells (36-38). This critical role of telomerase in tumor growth and development suggests that hTERT downregulation as a means of immune escape may itself have deleterious effects on tumor growth.

1.5 hTERT immunity vs. autoimmunity

A major concern of targeting hTERT immunologically is the potential lysis of rare normal cell types in which telomerase activity has been detected. Telomerase activity is absent in most major organs including heart, lung, liver, kidney, and brain, but activity or hTERT mRNA expression has been reported in hematopoietic stem cells, activated lymphocytes, basal keratinocytes, gonadal cells, and certain epithelial cells (39). However, hTERT-specific CTL *in vitro* do

not lyse either telomerase-positive CD34⁺ hematopoietic progenitor cells – with or without cytokine stimulation – nor activated T lymphocytes (4, 40, 41). It can only be speculated that these findings reflect either relatively low levels of hTERT protein, or alternatively, inefficient processing hTERT peptide in these normal cells. To be sure, activated B cells are susceptible to hTERT-specific lysis and notably represent the only cell other than tumor cells that to date have been demonstrated to undergo hTERT-specific lysis *in vitro* (4). However, in a recently completed cellular vaccine trial of 7 patients, no bone marrow toxicity or decreases in soluble immunoglobulin levels or B cell counts were noted after vaccination (7).

1.6 Prior human experience of hTERT peptide vaccination

In a Phase I clinical trial at the Dana-Faber Cancer Institute (led there by Dr. Robert H. Vonderheide, who has subsequently moved to the University of Pennsylvania as the PI of the current protocol), 7 HLA-A2 patients with advanced cancer (5 with prostate cancer, 2 with breast cancer) were vaccinated up to six times subcutaneously every other week with autologous monocyte-derived dendritic cells pulsed *ex vivo* with the hTERT HLA-A2 binding peptide I540 and keyhole limpet hemocyanin (KLH). A total of 34 vaccinations were administered from February 23, 2001 to October 11, 2001. There was one serious adverse event involving the development of grade 3 (NCI CTC) tumor pain from tumor progression, considered not related to the vaccine. All other adverse events were grade 2 or less, with most common events including transient fatigue (3 patients) and transient skin rash (2 patients, of which 1 patient developed 2 different skin rashes). All laboratory abnormalities were grade 2 or

less. Because hTERT is known to be expressed in normal bone marrow, bone marrow aspirates were evaluated pre- and post-vaccination, but no histological changes were observed post-vaccination compared to baseline. Similarly, given the potential toxicity of hTERT-specific CTL against activated B cells (as previously observed in vitro), serum immunoglobulin levels and absolute peripheral B lymphocyte counts were monitored, but no significant reductions were observed following vaccination.

Among six evaluable patients, one mixed clinical response was observed. This patient had metastatic breast cancer confined to multiple skin nodules on her chest wall that prior to vaccination, had progressed despite chemotherapy, radiation therapy, and hormonal therapy. Following vaccination, partial regression of some tumor nodules was observed without the appearance of new nodules or new sites of disease. Bidimensional measurement of the largest lesion demonstrated a 60% reduction. Biopsies of one lesion performed before and after vaccination demonstrated the induction post-vaccination of a mixed CD4+ and CD8+ lymphoid infiltrate into the tumor. Only poorly differentiated carcinoma without infiltrating lymphocytes were noted in the baseline biopsy. Four other evaluable patients (each with prostate cancer) had stable disease by standard radiographic assessment post-vaccination, and one (with breast cancer) had progressive disease.

Peptide/MHC tetramer analysis of peripheral blood mononuclear cells (PBMC) was used to track the induction of hTERT I540-specific CD8+ cells following vaccination. At baseline in all patients, no I540 hTERT tetramer+ CD8+ cells were found in PBMC stimulated in vitro for one week with hTERT I540 peptide or the negative control HTLV-1 L11 peptide. However, in the four

patients, a clear 1%-6% population of hTERT I540 tetramer+ CD8+ cells was identified following expansion during in vitro stimulation. This effect of the vaccine was peptide specific as post-vaccine PBMC stimulated in vitro with HTLV-1 L11 peptide (negative control) failed to stain with either hTERT I540 tetramer or HTLV-1 L11 tetramer. Multiple vaccinations were required for the induction of hTERT I540-specific CD8+ cells, as no tetramer+ cells were identified prior to the third or fourth vaccine.

PBMC were also evaluated by ELISPOT analysis to determine the cytokine-secreting capacity of hTERT-specific CD8+ cells induced after vaccination. For four patients, hTERT I540-stimulated PBMC markedly secreted IFN-gamma. Peptide specificity of the response was demonstrated in two ways: First, patient PBMC stimulated with hTERT I540 peptide did not secrete IFN-gamma when rechallenged during the ELISPOT analysis with HTLV-1 L11 negative control peptide. Second, PBMC stimulated with HTLV-1 L11 peptide did not secrete IFN-gamma whether rechallenged with HTLV-1 L11 or hTERT I540 peptide.

In summary, initial evaluation of patients vaccinated with dendritic cells loaded with hTERT peptide indicates the safety and feasibility of the approach. Importantly, immunity to hTERT was generated but no patient experienced side-effects attributable to autoimmunity directed against normal tissues that express telomerase, such as bone marrow, skin or lymph nodes.

1.7 hTERT as a widely expressed vaccine target for breast cancer immunotherapy

Telomerase activity has been reported in 90-95% of invasive breast cancers, and appears to be a significant factor in the biology of those cancers that express it (33, 42). Studies suggest that the overexpression of telomerase is an early step in the carcinogenic process (33). These findings of widespread expression support telomerase as a valid vaccine target in breast cancer. Furthermore, because of the critical role telomerase plays in tumor progression (35, 36), targeting hTERT therapeutically may also diminish the possibility of immune escape by decreased tumor expression of target antigens, because with hTERT, this can be expected to be detrimental to tumor growth (36). These basic science findings support the investigation of hTERT-directed vaccination for the treatment of human breast cancer.

1.8 Chemical adjuvants for antigen-specific cancer vaccines

Vaccination without adjuvant is rarely effective for the induction of specific immunity and can even result in the development of tolerance (43). Consequently, numerous adjuvants have been tested for antigen-specific cancer vaccines. For example, the inclusion of a chemical adjuvant with the subcutaneous injection of a melanoma-associated antigen has been used to achieve an intriguing degree of immunologic and clinical response in advanced melanoma patients (8). The mineral oil-based emulsifier and adjuvant, Montanide ISA 51 (Seppic, Inc.), has been particularly safe and effective in vaccine delivery for human cancer. It has been widely used, and a variety of reports show minimal toxicity and effective immunization (8, 21, 22, 27, 44-46), and thus we propose to use it here. Montanide ISA 51 is prepared for use as an emulsion with aqueous antigen solution. The surfactant for Montanide ISA 51 is

mannide oleate, a major component of the surfactant in incomplete Freund's adjuvants.

1.9 Use of GM-CSF in antigen-specific cancer vaccines

A second, and sometimes parallel approach to augment vaccination, has been the use of cytokines such as IL-2, IL-12, and (notable for this trial) GM-CSF. Subcutaneous injections of GM-CSF into the area of peptide injection will be used as an adjuvant strategy in this study. GM-CSF has a long track record of safety, both in the management of cytopenic patients (for which the drug is FDA approved) and in cancer vaccination. It was first used in a cancer vaccination trial over 6 years ago (47). Its properties as an activator of granulocytic and mononuclear cells makes it an excellent candidate for mobilizing dendritic cells, and may explain its apparent clinical activity. When given by subcutaneous or intramuscular injection it improved antibody responses to Hepatitis B vaccine both in healthy volunteers (48) and in patients with renal failure (49, 50). GM-CSF has also had extensive clinical use both as an adjuvant for peptide-based (11, 14, 16, 25, 28, 51, 52) and cell-based cancer vaccines (53). Clinical experience with recombinant human GM-CSF as an adjuvant has shown that it is very well tolerated, and can significantly enhance immune responses to vaccines in cancer patients (25)

1.10 Montanide ISA-51 and GM-CSF as a safe and effective adjuvant for cancer vaccines

In addition to extensive clinical experience using GM-CSF and Montanide ISA 51 separately, there are several reports of their use together (46, 54, 55). Combinations of GM-CSF and Montanide ISA 51 have been safe, with no

toxicity greater than grade two being observed using this combination. Two clinical trials in melanoma performed by the Eastern Cooperative Group have incorporated the combination of these agents in a pooled peptide vaccination schema (12). In mouse studies, GM-CSF was shown to be the single most effective cytokine (among 8 tested) for enhancing both cellular and humoral immunity to two previously characterized HIV-1 vaccine constructs formulated with adjuvant (56). The lack of toxicity and the likelihood of combining the benefits of both agents provides a rationale for the use of combining both agents here.

1.11 Utility of a viral-derived CTL epitope as a control peptide

Inclusion of viral-derived peptides been useful in early stage cancer vaccination strategies as a way of monitoring positive control peptide-specific responses. The nine amino acid peptide N495 (NLVPMVATV) derived from the pp65 protein of cytomegalovirus binds with extremely high affinity to HLA-A2 (57). As a recall antigen in most adults, this peptide can be used to generate cytotoxic CTL *in vitro* from the peripheral blood of nearly all seropositive donors (58, 59). The presence in peripheral blood of CTL specific for N495 peptide in HLA-A2 patients correlates with protection from CMV disease in a post-bone marrow transplant clinical setting (60).

This protocol incorporates the N495 CMV peptide to serve as an immunological control in two ways. First, in CMV seropositive patients, the generation of CMV-specific CTL following vaccination will serve as an important *positive* control for the simultaneous vaccination with hTERT peptide. Second, in seronegative patients, the potential generation of CMV-specific CTL

following vaccination will provide important immunological data with regard to patients' ability to respond to *neo* antigens. In contrast to T cells specific for CMV in seronegative individuals, however, T cells in cancer patients *may* in theory be tolerant to hTERT peptides, given potential prior presentation by overexpressing tumors and other normal cells. Consequently, a comparison of hTERT vs. CMV responses in seronegative vaccinated cancer patients will enable immunologic distinction between failure to overcome tolerance vs. failure to prime. For example, lack of CTL against hTERT but the demonstration of CTL against CMV would point to issues of hTERT-specific tolerance; lack of CTL against both hTERT and CMV CTL would suggest an inability of this vaccine formulation to prime naïve T cells in advanced cancer patients.

1.12 Pre-clinical and clinical studies supporting the rationale of this study

This trial is supported by a number of preclinical studies conducted by the PI and other investigators. These studies address both the immunogenicity of hTERT-derived peptide and CMV-derived peptide as a control immunogen. Major results of the studies are summarized below:

1. The hTERT-derived peptide I540 (ILAKFLHWL) binds with high affinity to HLA-A2 (4), the most common MHC class I allele with expression in nearly 50% of our cancer patients.
2. hTERT-I540-specific CTL generated in vitro from normal donors or cancer patients lyse hTERT+ tumor cells in an MHC-restricted fashion (4, 61, 62).
3. Mouse model systems confirm these observations. Anti-murine TERT immunity is elicited in mice vaccinated with dendritic cells transduced with murine TERT

RNA, leading to protection from tumor challenge in three separate models (63). Significantly, vaccinated mice remained healthy and did not suffer autoimmunity against hematopoietic cells or other murine tissues that express mTERT such as the liver (63).

4. A recently completed phase I trial of I540 peptide loaded onto autologous dendritic cells demonstrated the safety of this approach (7), as outlined in detail in Section 1.6.
5. The CMV-derived N495 (NLVPMVATV) binds with high affinity to HLA-A2 (57), and as a recall antigen in most adults, this peptide can be used to generate cytotoxic CTL from the peripheral blood of nearly all seropositive donors (58, 59).

1.13 Establishment of assays for clinical immunological assessment

This trial requires extensive immunologic evaluation of patient blood samples obtained before, during, and after scheduled vaccination. Routine assays necessary for this evaluation have been established by the investigators and include:

1. T cell tracking assays based on binding to HLA-A2/peptide tetramer complexes and monoclonal antibodies
2. T cell cytotoxicity assays for hTERT or CMV peptide expressing targets
3. ELISPOT assay for cytokine release from single peptide-specific T cells
4. Evaluation of tumor for telomerase activity and hTERT expression

1.14 Rationale for this study

This trial investigates the safety and possible therapeutic efficacy of targeting the telomerase reverse transcriptase hTERT in a peptide vaccine for advanced breast cancer patients. The rationale for this study, as reviewed in the above Sections, is founded on basic scientific findings of the investigators, our preclinical studies, one previous clinical trial by the investigators, and data previously reported by others in the literature. This rationale is summarized below:

1. hTERT can be targeted as a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes.
2. hTERT is highly expressed by >90% of all breast cancers but by few normal tissues.
3. A previous cellular vaccine using autologous dendritic cells expressing hTERT peptide was safe and feasible, and generated hTERT-specific CTL in the majority of patients.
4. Montanide ISA-51 and GM-CSF (separately and in combination) are safe and effective immune stimulants.
5. Peptide vaccination has been extremely safe and promising in a variety of other human cancer systems.

1.15 Rationale for addition of low-dose cyclophosphamide in an expanded cohort of vaccinated patients

In humans, a naturally occurring subpopulation of regulatory T cells constitutively expressing CD25 (the IL-2 receptor- α chain) comprises approximately 5-15% of peripheral blood CD4⁺ T cells (64). CD4⁺CD25⁺ Treg cells have recently been shown to inhibit autoimmune diabetes, prevent

inflammatory bowel disease, mediate transplantation tolerance, impede antitumor immunity and prevent the expansion of other T cells *in vivo* (65-68). In particular, human Treg cells markedly inhibit CD8 T cell activation (69). It is now clear that Treg contribute to immune dysfunction in cancer patients (66, 70, 71). Patients with breast cancer have been found to harbor a large number of regulatory T cells with potent immunosuppressive functions at the tumor site and in draining lymph nodes (72). In ovarian cancer, tumor-associated Treg have been shown to be associated with a high death hazard and reduced survival (71).

We hypothesize that low-dose cyclophosphamide will safely deplete CD4+CD25+ Treg cells from the peripheral blood of patients with breast cancer and may augment the immunogenicity of the proposed hTERT peptide vaccine. An expanded cohort of patients will receive cyclophosphamide prior to vaccination (see Section 5.14). Cyclophosphamide is extensively used for patients with breast cancer and is a cornerstone of chemoadjuvant therapy. Cyclophosphamide has had extensive previous testing as an agent that enhances the potency of immunotherapy. In mouse models, North presented evidence that cyclophosphamide enhances tumor immunotherapy and that the effect was independent of cytotoxic reductions in tumor burden (73). In other mouse studies, low-dose cyclophosphamide potentiated the anti-tumor effects of a GM-CSF-secreting breast tumor vaccine (74) or a vaccine of micropheres coated with tumor membrane vesicles (75). Cyclophosphamide also enhances the efficacy of adoptive transfer of antigen-specific lymphocytes (76, 77). The mechanism of these effects appears in part to be selective deletion or inhibition of tumor-induced suppressor or negative regulatory T cells (78, 79). In humans,

cyclophosphamide was shown to increase the response to a KLH vaccine in adjuvant (80), and in other studies, to augment cellular and humoral immunity in cancer patients (81, 82). In one phase II study of metastatic breast cancer patients, the use of low-dose cyclophosphamide prior to vaccination with a sTn-based vaccine resulted in improved survival (83). Doses required to achieve these immune-modulatory effects are lower than the standard dose of cyclophosphamide used for chemocytotoxicity and it appears to be important to deliver cyclophosphamide prior to vaccination (74, 84).

2 SPECIFIC AIMS

2.1 Primary Objective:

To determine the safety of vaccinating advanced breast cancer patients with increasing doses of hTERT I540 peptide administered subcutaneously with the adjuvant Montanide ISA 51 and the cytokine GM-CSF.

2.2 Secondary Objectives:

1. To assess the generation of hTERT peptide-specific vs. CMV peptide-specific CTL immunity as a result of vaccination
2. To determine the association, if any, between dose level and:
 - a. the generation of hTERT-specific CTL immunity
 - b. the development of hTERT-specific autoimmunity
3. To assess the effect of vaccination with hTERT I540 peptide on tumor response

3 SUBJECT SELECTION

Adult cancer patients meeting the following criteria will be eligible for enrollment in this study.

3.1 Inclusion criteria

Patients must meet the following criteria to be included in this clinical trial:

1. Stage IV (AJCC) breast carcinoma who have failed at least one conventional therapy for metastatic disease
2. Evidence of measurable or evaluable disease by clinical, radiographic, or laboratory assessment. (Use RECIST method for tumor assessment: measurable lesions must be at least one dimension (> 20 mm with conventional techniques or > 10 mm with spiral CT scan) or non-measurable lesions (all lesions < 20 mm by conventional techniques or ≤ 10 mm by spiral CT, and truly non-measurable lesions, i.e., pleural effusion, bone lesions, tumor markers).
3. Age > 18 years old
4. HLA-A2 positive by human leukocyte antigen typing
5. Baseline Eastern Cooperative Oncology Group (ECOG) Clinical Performance Status 0 or 1
6. Life expectancy > 6 months
7. Adequate hematologic function established within 14 days before first vaccination:
 - i. WBC > 3.0
 - ii. Plt > 75
 - iii. Hgb > 10 g/dl
8. Adequate renal function established within 14 days before first vaccination defined as serum creatinine < 1.5 times upper limit of normal

9. Adequate hepatic function established within 14 days before first vaccination defined as:
 - i. Total bilirubin < 1.5 times upper limit of normal, and
 - ii. ALT and AST < 2.5 times upper limit of normal
10. Contrast CT and/or MRI of the brain negative for central nervous system metastases within 30 days before first vaccination
11. Women of child bearing potential must have a negative pregnancy test (blood or urine) within 14 days before first vaccination and agree to use appropriate contraception from study screen through the duration of the trial. Men must agree to use appropriate contraception from study screen through the duration of the trial.
12. Signed and dated written informed consent

3.2 Exclusion criteria

Patients who meet any of the following exclusion criteria are not eligible for the study:

1. History of brain metastases within the last four years
2. Positivity for HIV-1/HIV-2, HTLV-1, Hepatitis B virus, and Hepatitis C virus
3. Active infection
4. The use of the following within 30 days before the first vaccination:
 - i. chemotherapy
 - ii. radiation therapy
 - iii. immunosuppressive drugs
 - iv. systemic glucocorticoids
 - v. hematopoietic growth factors

- vi. experimental therapy
- 5. Use of anti-coagulants such as coumadin, heparin, or Lovenox within 14 days before the first vaccination, with the exception of low dose anti-coagulants to maintain intravenous catheter patency
- 6. Initiation of hormonal agent (such as tamoxifen, anastrozole, or letrozole) in the 30 days before the first vaccination. Patients who have been on a hormonal agent for at least 30 days prior to first vaccination with progressive or stable disease are permitted to enroll, but required to stay on this hormonal agent for the duration of the study.
- 7. Initiation of immunotherapy (such as trastuzumab-Herceptin) in the 30 days before the first vaccination. Patients who have been on trastuzumab for at least 30 days prior to first vaccination with progressive or stable disease are permitted to enroll, but required to stay on trastuzumab for the duration of the study.
- 8. History of bone marrow or stem cell transplantation (allogeneic or autologous)
- 9. Pregnant women or nursing mothers
- 10. History of alcohol abuse or illicit drug use within 12 months of study initiation
- 11. Clinically significant comorbid disease or other underlying condition, including major autoimmune disorders that would contraindicate study therapy or confuse interpretation of study results
- 12. Significant psychiatric disorder and any other reason in the Investigator's opinion that would jeopardize protocol compliance or compromise the patient's ability to give informed consent

4 SUBJECT ENROLLMENT

Patients identified as being appropriate for this trial will be referred to one of the clinical investigators for evaluation. During the initial evaluation, participation in this study as well as alternative treatment options will be discussed. The risks and benefits of the proposed therapy will be explained and discussed with the patient. The patient will be asked to review and sign the written study consent. As many as 3 cohorts of 5 to 8 patients each will be enrolled, depending on toxicity. Additional patients will be treated at the maximum biological dose (MBD) so that a total of 16 patients (including those in the original dose level cohort) will be treated. So far we have enrolled six subjects at dose level one, seven subjects at dose level two, and five subjects at dose level three. A total of nine additional subjects at the dose level two are to be enrolled. We anticipate a maximum of 27 patients may be treated.

5 STUDY PROCEDURES

Study procedures are also outlined in the protocol schema (see Figure 6.1 below) and Table 2 (also below).

5.1 HLA typing

Patients will be typed for MHC class I alleles. Only those individuals who are HLA A2 positive are eligible for the study.

5.2 Tumor biopsy/body fluid specimens

In patients with tumors involving the skin, subcutaneous tissue, subcutaneous lymph nodes, and/or body fluids (such as pleural effusions and ascites), a biopsy or procedure to obtain tumor cells before vaccination may be performed. Part of the tumor or fluid sample will be directly transported to the Principal Investigator's laboratory for processing and evaluation, including analysis of hTERT expression and tumor lymphocytic infiltration. If fresh tumor or body fluid cannot be obtained or if a biopsy or procedure is not clinically possible or appropriate, tumor evaluation may be attempted by analysis of available archival pathologic material under the direction of the Principal Investigator. Although such a biopsy is felt to be important for immunologic assessment, it is not required for enrollment.

5.3 Screening

The following evaluation will be performed prior to enrollment to establish patient eligibility. All tests will be required within 14 days prior to first vaccination. Viral studies (HIV, HCV serology, and HBV surface antigen, surface antibody, and core antibody) will be performed within 30 days from first vaccination. Tumor biopsy and HLA typing can be performed at any time preceding vaccination.

1. Medical history including prior treatment, current medicines, and drug allergies
2. Physical exam including blood pressure, pulse, temperature, respiration, weight, and ECOG performance status
3. Laboratory studies comprising CBC/diff, Cr, AST, ALT, and total bilirubin
4. Viral studies comprising HIV-1, HIV-2, HTLV-1, and HCV serology and HBV surface antigen, surface antibody, and core antibody
5. Signed, informed consent

Table 2. Schedule of Evaluations and Measurements

ACTIVITY	Pre Trtmt Period	72 hrs prior to V1	V1	6-8 days after V1	V2	V3	V4	V5	V6	V7	V8	End of study ^b
Registration	X											
Informed Consent Signed	X											
Medical History	X		X		X	X	X	X	X	X	X	X
Physical Examination	X		X		X	X	X	X	X	X	X	X
Vital Signs	X		X		X	X	X	X	X	X	X	X
HLA Typing (if unknown)	X											
CBC/diff, Cr, AST, ALT, total bilirubin	X											
CBC/diff		X		X								
Viral Testing	X											
Leukapheresis	X											X
CMV serology			X									X
Pregnancy Test, if applicable	X											
Tumor staging	X						Between V4 and V6					X
Tumor Biopsy (if feasible)	X						Any time after V3					
CBC/diff, chemistries (OCOMP)			X		X	X	X	X	X	X	X	X
Urinalysis			X									
Tumor markers			X				X			X		X
ECG			X									
Additional tests ^a			X									X
Immunology samples (first 3 cohorts)			X ^{c,e}				X ^f	X ^f		X ^f		X ^{d,e}
Immunology samples (MBD extension cohort)		X ^{c,e}	X ^h	X ^h	X ^h		X ^f	X ^f		X ^f		X ^{d,e}
Vaccination			X		X	X	X	X	X	X	X	
Cyclophosphamide		X ^g										
Adverse event monitoring		X	X	X	X	X	X	X	X	X	X	X

^a PT/PTT, TSH, and quantitative immunoglobulins.^b Within 30 days of final vaccine.^c Not done if patient undergoes baseline leukapheresis^d Not done if patient undergoes end-of-study leukapheresis

e 75 ml of blood drawn in green top tubes, transported at room temperature to Dr. Vonderheide, 551 BRB
f 50 ml of blood drawn in green top tubes, transported at room temperature to Dr. Vonderheide, 551 BRB
g Cyclophosphamide 300 mg/m² iv
h 30 ml of blood drawn in green top tubes, transported at room temperature to Dr. Vonderheide, 551 BRB

5.4 Tumor assessment and staging

Patients will undergo complete clinical and radiographic evaluation necessary to establish disease stage and to document the full extent of measurable disease. This evaluation will include at least a CT of chest/abdomen/pelvis, bone or PET scan, and a contrast CT or MRI of the brain. All tests will be performed as close to the day of first vaccination as possible but at least within 30 days before first vaccination. Appropriate tumor markers for the patient's disease will be obtained prior to first vaccination.

5.5 Leukapheresis

Within 14 days of screening assessments and after study enrollment, patients will undergo leukapheresis at the University of Pennsylvania Blood Bank according to standard clinical procedures. Peripheral blood leukocytes will be transported to the Principal Investigator's laboratory at the University of Pennsylvania and used for immunoassessment research assays. Although baseline leukapheresis is felt to be important for the aims of this study, a patient's inability to undergo leukapheresis for clinical reasons, e.g. lack of adequate venous access, will not disqualify the patient from receiving vaccination.

5.6 Baseline clinical assessments

The following evaluation will be performed on the day of first vaccination.

1. Medical history and physical exam, including vital signs
2. Laboratory chemistries comprising CBC/diff, OCOMP, PT/PTT, TSH, quantitative immunoglobulins, and CMV serology.

3. Urinalysis
4. ECG
5. Tumor markers as deemed appropriate by the clinical investigator
6. Immunologic studies as noted in Table 2.
7. Adverse event assessment

5.7 Grading of toxicity

Toxicity will be graded using the NCI Common Toxicity Criteria (see <http://ctep.info.nih.gov/reporting/ctc.html>).

5.8 Vaccine preparation

Vaccine components

- Peptides were synthesized under good manufacturing practices (GMP) by Clinalfa (Laufelfingen, Switzerland) and vialled as a sterile lyophilized powder without preservatives. The peptides used in this study are: (i) hTERT I540 with the amino acid sequence of ILAKFLHWL and (ii) CMV N495 with the sequence of NLVPMVATV.
- Montanide ISA 51 adjuvant is produced by Seppic, Inc. (Fairfield, NJ) under good manufacturing practice. It is a mineral oil solution based on mannide oleate which is used to emulsify the peptides.
- Recombinant human GM-CSF (Sargramostim, Immunex Corp.) is a clinical grade, FDA-approved drug and will be supplied by Hospital of the University of Pennsylvania pharmacy. It will be administered as 70 µg subcutaneously in the same area as the two peptide/Montanide ISA 51 injections given per vaccination.

Vaccine formulation and administration.

Vialed peptide will be kept frozen at the research pharmacy of the Hospital of the University of Pennsylvania until immediately before reconstitution. Each of the two peptides will be reconstituted separately in the pharmacy with Sterile Water for Injection, USP, to make two peptide solutions. Using empty sterile vials for each of the two peptides, a final 1.0 ml volume of peptide solution mixed in Montanide ISA 51 will be prepared by aliquoting appropriate volumes of peptide solution and Montanide ISA 51 (as described in Table 3). These two peptide solutions (one each for hTERT I540 and CMV N495) will then be transported to the clinical unit and emulsified by vortexing the vial for 12 minutes. The entire contents of a vial will then be immediately withdrawn and injected subcutaneously in a lateral thigh. The hTERT I540 peptide/Montanide ISA 51 emulsification will be given in the right thigh and the CMV 495 peptide/Montanide ISA 51 emulsification will be given in the left thigh. GM-CSF will then be administered as two separate 70 µg injections in the subcutaneous tissues in the same regions where the peptide/Montanide ISA 51 solutions were given.

Table 3. Peptide injections will be prepared for injection as follows

Dose Level	Peptide Dose	Peptide dilution concentration	Peptide dilution Volume	Montanide ISA 51 Volume	Final Dose Volume
1	10 µg	20 µg/ml	0.5 mL	0.5 mL	1.0 mL
2	100 µg	200 µg/ml	0.5 mL	0.5 mL	1.0 mL
3	1000 µg	2000 µg/ml	0.5 mL	0.5 mL	1.0 mL

5.9 Vaccination schedule and route

A total of eight vaccinations will be given over 27 weeks. Each vaccination will require two injections of peptide/Montanide ISA 51 (one per thigh) and two injections of GM-CSF (one per thigh). If necessary due to injection site reactions from previous vaccinations, injections can be given in the upper arms near the deltoid region or in the buttocks, based on the clinical judgment of study investigators but in each case, hTERT I540 peptide will be given a right-sided fashion and CMV 495 peptide in a left-sided fashion. The intervals between injections will be 2 weeks (+ 3 days) for the first four injections, 4 weeks (+ 3 days) for the next three injections, and 8 weeks (+ 3 days) between the seventh and eighth injections (see Figure 6.1). Vaccinations will be given in the outpatient clinic of the Hospital of the University of Pennsylvania. After the first vaccination, patients will be observed for at least 1 hr including measurement of vitals signs prior to discharge. With subsequent vaccinations, patients may be discharged immediately following injection.

5.10 Vaccination postponement criteria

Please see section 5.11.

5.11 Vaccination termination and modification

Patients will be terminated from the study for any of the following reasons:

1. Any treatment related grade 3 or higher toxicity
2. Postponement of vaccination beyond 30 days from the original day the vaccination was scheduled to be administered.
3. Three separate postponements of vaccinations. For example, a 1-day to 29-day postponement of vaccination number three and a 1-day to 29-day postponement of vaccination number four would be allowed in a single patient, but an additional 1-day to 29-day postponement of vaccination number five would result in study termination.
4. Significant deviation from the treatment and evaluations outlined in the protocol including use of unapproved concomitant medicines.

Given the phase I nature of this protocol, most patients are expected to have progressive disease at study entry. In cases of successful T cell-mediated immunotherapy (e.g. DLI following allogeneic BMT for relapsed CML) a lag period of weeks to months is required to achieve optimal clinical response. Furthermore, in study patient #004, radiographically apparent "progression" as defined by the RECIST criteria corresponded pathologically to tumor necrosis and inflammation. After four vaccines, chest CT demonstrated 2 stable pulmonary nodules and a stable breast mass. After eight vaccines, these lesions were >20% larger, meeting RECIST criteria for progression. However, a biopsy of the dominant breast mass revealed that 50-80% of the mass was now necrotic with associated inflammation. For these reasons, we do not feel that radiographically defined disease progression is a reason in itself for vaccine

termination. The clinical investigators will use clinical judgment to determine in consultation with the patient whether a study patient, should switch to other available therapeutic options, if any, or best supportive care." Any patient with rapidly and/or seriously advancing disease will be removed from study and other options discussed.

5.12 Treatment cohorts

Patients will be treated in cohorts of 5 or 8 patients (depending on observed toxicity) (see Section 5.14). Each patient in a given cohort will receive the same dose of hTERT I540 and CMV N495 peptides. Three dose levels will be tested, based on the amount of peptide in each injection (ranging from 10 µg to 1000 µg) (Table 4). Cohorts will be treated sequentially.

The same dose of hTERT I540 peptide and CMV N495 peptide will be given for each vaccination. No individual patient will be treated with escalating doses. No dose reduction will be permitted *except* if the MTD is determined during active treatment of patients to be less than the current dose level. In this case, patients will be treated with the MTD for any further scheduled immunizations.

Table 4. Treatment dose levels and cohorts

Cohort	Number of patients	Treatment	Dose of each peptide used	GM-CSF at each peptide injection site
1	5-8	Right extremity: hTERT I540 peptide emulsified in Montanide ISA 51 s.c. Left extremity: CMV N495 peptide emulsified in Montanide ISA 51 s.c.	10 µg	70 µg s.c.

2	5-8	Right extremity: hTERT I540 peptide emulsified in Montanide ISA 51 s.c. Left extremity: CMV N495 peptide emulsified in Montanide ISA 51 s.c.	100 µg	70 µg s.c
3	5-8	Right extremity: hTERT I540 peptide emulsified in Montanide ISA 51 s.c. Left extremity: CMV N495 peptide emulsified in Montanide ISA 51 s.c.	1000 µg	70 µg s.c

5.13 Dose limiting toxicities

Patients will be evaluated for dose-limiting toxicity (DLT) from the day of first vaccination to the third vaccination. DLT is defined as “any treatment related (i) grade 3 or higher hematologic or non-hematologic toxicity; (ii) grade 2 or higher autoimmune reaction; or (iii) grade 2 or higher allergic reaction or reaction that involves bronchospasm or generalized urticaria, as determined by the Data Safety Monitoring Board (DSMB) (See section 10.7).

5.14 Accrual to next dose level and maximum tolerated dose

Accrual of patients and escalation to the next dose level will occur based on a 5-8 rule as follows:

- If 0/5 patients at a dose level experience DLT, patients will be accrued to the next dose level
- If >2/5 patients at a dose level experience DLT, then dosing at that level will be stopped and the preceding dose level will be considered the MTD
- If 1/5 patients at a dose level experience DLT, then 3 additional patients will be treated at the level.
If 0/3 of the additional patients experience DLT, then patients will be accrued to the next dose level; otherwise, dosing at that level will be stopped and the preceding dose level will be considered the MTD

Differentiating MTD from maximum biological dose.

The MTD may or may not be the maximum biological dose (MBD). The MBD will be determined based on consideration of clinical response data, pathological response data, or immunological response data. The principal investigators and the sponsor, with input from the DSMB, will make this determination and the IRB, CTSRMC, and FDA will be informed regarding this determination. The MBD cannot be deemed to be higher than the MTD.

Enrollment of additional patients at the MBD

Additional patients will then be treated at the MBD so that a total of 16 patients (including those in the original dose level cohort) will be treated at the MBD. If a patient fails to complete at least three vaccinations for reasons other than a DLT (e.g. due to progressive disease requiring other treatment), they will be replaced in the cohort. Stopping rules for enrollment of patients in the expanded cohort at the MBD are noted in Section 6.2 based on toxicity.

Addition of low-dose cyclophosphamide preconditioning at the MBD.

In addition to receiving vaccination on the schedule noted in Figure 6.1, patients in the expanded MBD cohort will receive low-dose cyclophosphamide 3 days prior to the first vaccination. The dose to be delivered is 300 mg/m^2 in 150 mL NS intravenous over 30 minutes. This strategy tests the hypothesis that hTERT-specific vaccination in combination with cyclophosphamide in breast cancer patients can down modulate regulatory T cells and safely induce anti-hTERT

CTL immune responses, with the rationale for this approach detailed in Section 1.15.

5.15 Interim evaluations and vaccinations (see Table 2)

At the time of each subsequent vaccination, patients will be evaluated with:

1. Medical history, including adverse event evaluation
2. Physical exam, including vital signs and performance status
3. Laboratory studies as noted in Table 2.
4. Immune studies as noted in Table 2.
5. Adverse event assessment

At any time between the *fourth and sixth* vaccination, tumor assessment and staging will be performed, including clinical and radiographic evaluation necessary to assess measurable or non-measurable disease.

During these interim evaluations, injection sites may be biopsied if warranted by the severity of the inflammatory reaction.

Additional blood draws as noted in Table 2 will be obtained from patients treated with cyclophosphamide on the MBD extension cohort in order to determine the effect of cyclophosphamide on immune cells.

5.16 Study completion and evaluation

The following evaluation will be performed within 30 days after the last vaccination:

1. Medical history and physical exam, including vital signs and performance status
2. Laboratory studies as noted in Table 2.
3. Adverse event assessment
4. Tumor assessment and staging, including clinical and radiographic evaluation necessary to assess measurable or non-measurable disease. Patients will be considered evaluable for response if at least three vaccines are administered
5. Tumor markers as deemed appropriate by the clinical investigator

Any time up to 60 days after the last vaccination, patients will undergo leukapheresis at the University of Pennsylvania Blood Bank according to standard clinical procedures. Peripheral blood leukocytes will be transported to the Principal Investigator's laboratory for immunological studies (Section 5.21). If a patient can or does not undergo leukapheresis, a 75 ml blood sample will be drawn at the end-of-study visit to be used for immunological studies.

5.17 Long-term follow-up

All patients will be contacted by the primary oncologist or clinical investigators via clinic visit or telephone at 6 and 12 months after the last vaccination to determine survival status and disease status.

5.18 Concomitant medicines and therapy

The use of glucocorticoids, immunosuppressive drugs, chemotherapy, radiation therapy, hematopoietic growth factors, or other investigational products are not

permitted until study completion and evaluation. The use of trastuzumab, hormonal agents (such as tamoxifen), and anti-coagulants will be permitted only as detailed in Section 3.2.

5.19 Response criteria

Tumor response will be evaluated using RECIST criteria.

- Complete response (CR): Disappearance of all disease
- Partial response (PR): A reduction of greater than 30% in the sum of the longest diameter (LD) of all measurable lesions, taking as reference the baseline sum LD
- Progressive Disease (PD): An increase of greater than 20% in the sum of the LD of all measurable lesions, taking as reference the smallest sum LD at baseline, OR clear cut increases in the magnitude of non-measurable lesions, OR the appearance of new lesions
- Stable Disease: Neither sufficient decrease to qualify for PR or CR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD at baseline

5.20 Post-vaccination tumor biopsy and evaluation.

Biopsy of select lesions after cancer vaccination provides some of the most important data for biologic and immunologic assessment of patient response. Furthermore, histologic evaluation of effector cell infiltration and tumor destruction provides important data for subsequent vaccination protocols. In patients with tumors involving the skin, subcutaneous tissue, or subcutaneous lymph nodes, and/or body fluids (such as pleural effusions and ascites), a biopsy

or procedure to obtain tumor cells under local anesthesia may be performed after vaccination. Part of the tumor sample obtained will be directly transported to the principal investigator's laboratory for evaluation, and the rest will be sent to Pathology. Although such biopsies and procedure would ideally be performed after completion of all eight immunizations, they may be performed at any time after three immunizations.

5.21 Immunologic Endpoints

Immunologic monitoring in this trial will be extensive and involve the assays described below. These assays will be performed from patient samples obtained at baseline, during vaccination, and at study completion. Tumor samples will also be used to monitor immunologic effects of this vaccine at times before and after vaccination. Responses against hTERT-I540 will be compared to those against CMV N495. A chief aim of this trial is to perform the following immunologic evaluation of vaccinated patients:

1. Direct enumeration of peptide-specific cytotoxic T lymphocytes (CTL) using peptide/HLA-A2 tetramers in flow cytometry
2. Determination of CTL cytotoxicity using standard cytotoxicity assays with targets including peptide-pulsed cells, wild-type and antigen-transfected cell lines, and (when available) autologous tumor
3. Determination of cytokine production of single antigen-specific cells using ELISPOT or intracellular cytokine technology.
4. Determination of telomerase expression and immune phenotype of autologous tumor using biopsy and procedure samples.
5. Determination of Treg frequency and function using standard in vitro assays.

6 BIOSTATISTICAL ANALYSIS

6.1 Dose escalation

This phase I study seeks to treat 5 patients at each of 3 dose levels of hTERT I540 peptide, with additional patients added if necessary according to a 5-8 rule as follows:

- If 0/5 patients at a dose level experience DLT, patients will be accrued to the next dose level
- If $>2/5$ patients at a dose level experience DLT, then dosing at that level will be stopped and the preceding dose level will be considered the MTD
- If 1/5 patients at a dose level experience DLT, then 3 additional patients will be treated at the level.
If 0/3 of the additional patients experience DLT, then patients will be accrued to the next dose level; otherwise, dosing at that level will be stopped and the preceding dose level will be considered the MTD

The probability of dose escalation for a given true toxicity is given below for the 5-8 rule:

True toxicity rate	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Probability of dose escalation	0.83	0.54	0.29	0.13	0.05	0.015	0.003	<0.001	<0.001

6.2 Additional patients treated at the MBD

Additional patients will be treated at the MBD so that a total of 16 patients (including those in the original dose level cohort) will be treated at the MBD. The addition of patients at the MBD will further improve the estimation of

toxicity at the MBD and also increase the precision of immunoassessment measurements (see Section 6.3).

Patients in this expanded cohort at the MBD will be clinically evaluated for evidence of toxicity. Data obtained after the treatment of every three patients will be reviewed, and the Bayesian posterior probability that the grade 3 or 4 treatment-related toxicity rate exceeds the maximal tolerated level of 20% will be calculated. If this posterior probability exceeds 80%, termination of enrollment will be considered. Assuming a uniform prior on the toxicity rate (equivalent to 1 event in two prior patients), termination would be considered with two treatment-related grade 3 or 4 toxicities in the first 6 subjects, 3 in the first 9, 4 in the first 12, and so on. With 10 patients, there is at least 90% power for detecting any previously unexpected toxicity whose prevalence in this population is at least 21%.

If the MTD is determined during active treatment of patients to be less than the current dose level, patients will be treated with the MTD for any further scheduled immunizations.

6.3 Precision for primary and secondary endpoints

Statistical considerations for the evaluation of secondary endpoints have been calculated based on peptide/MHC tetramer analysis which provides a simple, quantitative measurement of specific T cell precursors before and after vaccination. Given that baseline (pre-vaccination) T reactivity is below the level of tetramer detection ($<0.02\%$ of CD8+ T cells), tetramer analysis post-

vaccination yields a binary result regarding “immune response” if a cutoff for positivity is set (i.e., 0.10% of CD8+ cells based on the principal investigator’s previous hTERT vaccine study). 95% confidence intervals for immune response is given below for 5 patients in a cohort and for 12 patients at the MTD. We note, in addition, that the same calculations of confidence intervals apply to the estimation of toxicity rates.

# of responses out of 5 patients	Exact 95% confidence intervals
0	0.00, 0.52
1	0.005, 0.72
2	0.05, 0.85
3	0.14, 0.95
4	0.28, 0.99
5	0.48, 1.0

# of responses out of 12 patients	Exact 95% confidence intervals
0	0.00, 0.26
1	0.002, 0.38
2	0.02, 0.48
3	0.05, 0.57
4	0.10, 0.65
5	0.15, 0.72

6	0.21, 0.79
7	0.28, 0.85
8	0.35, 0.90
9	0.43, 0.95
10	0.52, 0.98
11	0.62, 0.99
12	0.74, 1.00

6.4 Biostatistical analysis of patients treated with cyclophosphamide

Three previous measurements of the average frequency of CD4+CD25+ T cells in PBMC of carcinoma patients are available. One is a published study of 35 breast cancer patients (72); a second is a published study of 42 patients with carcinoma (not necessarily breast cancer) (85); and the third is our preliminary evaluation of the breast cancer patients already enrolled and treated in this phase I study. The average frequency of CD4+CD25+ T cells in patients (mean + SD) was 16.6% + 7.1%, 12.5% + 5.8%, and 13.3% + 8.3%, respectively. Averaging the variances, we get an estimated SD of 6.8%. We will test the null hypothesis that cyclophosphamide has no effect at any post-treatment time-point on CD4+CD25+ T cells versus the alternative that it causes CD4+CD25+ percentage to decline, using a paired t test on the pre- and post-treatment percentages. For the MBD expansion cohort, the power to detect a 50% decrease in the mean percentage comparing pre- to post-cyclophosphamide is at least 80% in a two-tailed test at the .05 significance level.

7 RISKS AND DISCOMFORTS

7.1 Venipuncture

Patients may experience slight discomfort at the site and rarely develop an ecchymosis or superficial thrombosis.

7.2 Leukapheresis

Potential adverse reactions related to leukapheresis include:

1. Exacerbation of symptoms from an underlying medical condition including migraine headache, asthma/emphysema, or cardiovascular disease.
2. Paraesthesias, dizziness, mild chest tightness, or cough related to the reinfusion of autologous red blood cells treated with citrate
3. Ecchymoses and infection at catheter sites
4. Hypotension, hypertension, or bradycardia from citrate toxicity
5. Allergic reactions
6. Anemia, if red blood cells cannot be reinfused

7.3 Vaccination with peptide, GM-CSF, and Montanide ISA 51

Subcutaneous delivery of peptide with GM-CSF and/or Montanide ISA 51 has been well tolerated in previous trials without significant side-effects. However, some patients may experience fever, or injection site reactions including erythema, induration, pain, tenderness, and ecchymoses. GM-CSF may also cause bone pain. In the first 16 patients treated thus far in this trial, there have been no treatment-related SAEs or DLTs. The most common side effect has been grade 1 or 2 injection site reactions, which include erythema, induration, pruritus, pain, and/or ecchymoses. Other side effects include transient fever and chills,

fatigue, generalized achiness, and pain or pruritus in areas where the tumor is present.

7.4 Immunologic response to CMV N495 peptide

No side-effects are expected if patients develop an immune response to the CMV N495 peptide as a result of vaccination. This type of response is part of the natural, readily detectable response to CMV in HLA-A2 healthy individuals and causes no disease or pathology. Indeed, the presence in peripheral blood of CTL specific for N495 peptide in HLA-A2 patients recovering from stem cell transplantation correlates with protection from CMV disease (60).

7.5 Immunologic response to hTERT peptide

The risk of an adverse reaction due to vaccination with hTERT is not fully known. Data from our phase I trial using hTERT pulsed dendritic cells suggests that despite the generation of immunity, there is no evidence of autoimmunity (Section 1.6). However, further assessment of toxicity related to immunologic response to hTERT peptide is one of the aims of this study. The development of anti-hTERT immunity as a result of vaccination would not be expected to cause side-effects in organ systems that lack hTERT expression such as adult cardiac, renal, hepatic, pulmonary, neural, skeletal, and adipose tissues. hTERT and/or telomerase activity has been reported in a few normal cell types including a fraction of hematopoietic stem cells and precursors, basal keratinocytes, activated T and B lymphocytes, gonadal cells, and rare epithelial cells. In most of these cell types, hTERT expression is felt to be low. In the consideration of potential hTERT-specific cytotoxicity of normal, telomerase-positive cells, preclinical studies

failed to demonstrate such cytotoxicity against CD34-enriched hematopoietic cells or activated T cells.

7.6 Skin biopsy

Those patients undergoing biopsy of injection sites may experience minor bleeding, erythema, and pain, and less likely, infection.

7.7 Tumor biopsy

Biopsies and procedures necessary to obtain tumor tissue before and potentially after vaccination may cause bleeding, pain, and infection. Exact risks and potential side-effects will be described to the patient by the surgeon or operator, depending on the type of biopsy and procedure recommended and separate consent will be obtained for each of these procedures.

7.8 Cyclophosphamide

At the dose proposed for this study (300 mg/m²), side-effects from cyclophosphamide are anticipated to be few and mild. However, at higher doses, the drug can cause nausea, vomiting, alopecia, bone marrow suppression, SIADH, bladder toxicity, and rash. Cyclophosphamide is commercially available.

8 **POTENTIAL BENEFITS**

As discussed in Section 1, there is a growing clinical experience with antigen-specific vaccination and meaningful clinical responses are now being reported from studies in a few types of malignancies. Notable among these have been

clinical responses achieved in malignant melanoma and B cell lymphoma in which efficacy was linked to detectable specific immune responses. It is possible that some patients in this study will have tumor reduction based on immune reactivity against hTERT-I540.

Data generated from this trial will assist in the design of other antigen-specific immunotherapies and will provide further assessment of hTERT as a widely applicable target for such approaches. Fundamental questions remain concerning optimal immunogens, adjuvants, dose schedules, and vaccine routes that elicit clinically meaningful anti-tumor immunity. This study seeks to address some of these issues and provide a foundation for future studies in antigen-specific immunotherapy.

9 ADVERSE EVENTS

9.1 Adverse events

Adverse event is defined as any untoward medical occurrence in a patient participating in this clinical study who was administered the vaccination. An AE does not necessarily have to be causally related to the product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with vaccination, whether or not considered related to the vaccination. The Investigator will determine if abnormal laboratory values are AE. AE intensity will be recorded based on NCI CTC toxicity criteria.

9.2 Serious adverse event definitions, documentation, and reporting

Serious adverse events (SAE) will be reported to the Internal Review Board and to FDA as dictated by current institutional and federal requirements. In addition, SAE must be reported immediately to Dr. Robert H. Vonderheide, the sponsor, at 215-573-4265. It is the responsibility of the principal investigator to notify the IRB and the sponsor. In turn, the sponsor will notify other regulatory bodies, e.g. FDA, as required, of any serious adverse event (including death) that occurs during the course of this study. In accordance with 21 CFR 312.32, the investigator shall provide a written report of any AE associated with the use of the drug that is both serious and unexpected as soon as possible but no later than by 15 calendar days. Any AE that is unexpected fatal or life-threatening associated with the use of the drug must be reported as soon as possible but no later than by 7 calendar days by telephone or FAX with a complete written report as soon as possible within 8 additional calendar days. Any AE event that occurs during the study must be treated by established standards of care and followed until the event has resolved or until the condition has stabilized.

SAE include medical occurrences that are identified from the time of first vaccination to 30 days after the last vaccination. SAE is any untoward medical occurrence that at any dose

- Results in death;
- Is life-threatening (the patient was, in the Investigator's opinion, at immediate risk of death from the event);
- Requires inpatient hospitalization or prolongation of existing hospitalization;

- Results in persistent or significant disability/incapacity (substantial disruption of ability to conduct normal life functions);
- Is a congenital anomaly/birth defect; or
- Is an important medical event (an event that may not fit the other criteria for an SAE listed above, but may jeopardize the patient or may require intervention to prevent one of the outcomes listed above)

9.3 Unexpected AE

For the purposes of this study, which includes a drug not yet approved for marketing, a related AE may be considered unexpected when the nature or severity of the reaction is not consistent with the current understanding of the drug.

9.4 Causality

- Not Related: An adverse event that is definitely produced by the patient's clinical state or by other modes of therapy administered to the patient
- Related (Definitely, Probably, Possibly): Follows a reasonable temporal sequence from study drug administration. Could have been produced by the subject's clinical state or by modes of therapy.
- Unknown: Relationship of the adverse event to the study drug cannot be determined.

A concurrent illness is defined as any AE or pathological condition of known etiology that is considered unrelated to study drug. Any illness that develops during the course of the study should be recorded on the case report form (CRF).

9.5 Monitoring of Adverse Events

Patients will be monitored and reported for AE throughout the study, until 30 days after the last vaccination or the time of initiation of a new cancer therapy, whichever comes first. Medical events that occur between the signing of the informed consent form and the first intake of any study drug will be documented on the Medical History page of the CRF. Adverse events will be monitored both at the time of vaccinations and afterwards. Any symptoms that occur post-injection will be recorded.

The Clinical Investigator will assess the patient for AE at each study visit. The patient will be instructed to notify the Investigator of any AE occurring between study visits. Patients will be given the name and telephone number of the clinical professional who will assess the complaints and arrange for appropriate medical evaluation. All AE will be recorded in standard medical terminology on the AE page of the CRF. Each AE will be evaluated for seriousness, intensity, duration, and relationship to study drug. Any treatment provided for the event will be recorded. The intensity and causality of AE will be assessed by the Investigator and recorded on the CRF. SAE and pregnancies will be recorded on the Adverse Event form of the CRF and on the Serious Adverse Event form.

The principal investigator will also oversee the handling and processing of clinical samples used in the research laboratory and will supervise the immunologic assessments described in Section 5.21.

9.6 Exposure in utero

If any patient becomes or is found to be pregnant while receiving vaccinations or within 4 weeks of discontinuing vaccination, the Principal Investigator will be immediately informed. If the outcome of the pregnancy meets the criteria for classification of SAE, the PI must report this to the IRB and FDA as required by institutional and federal regulations.

10 QUALITY ASSURANCE

The study will be conducted in accordance with the Code of Federal Regulations and the guidelines of the International Conference on Harmonisation (ICH).

10.1 Consent Form and Patient Information

The patient will sign an Informed Consent form before any study-related procedures are performed. The Investigator will provide the patient with oral and written information related to the study. The aims of the study, study procedures, potential risks, discomforts, and expected benefits will be discussed. The patient will be informed and agree that study data will be accessible to the clinical investigators and possibly to Health Authority personnel. Patients will be informed that participation is voluntary and that the patient has the right to withdraw from the study at any time.

10.2 Institutional Review Board

Prior to commencement of the study, the protocol and any amendments, and the informed consent form will be submitted to the IRB. Written approval of the protocol and the informed consent form will be obtained from the IRB prior to commencement of the study. Protocol amendments will be reviewed and

approved by the IRB according to the Board's usual procedure. It is the responsibility of the Investigator to obtain approval of these documents from the IRB. The Investigator will maintain an accurate and complete record of all documents submitted to the IRB. The records will be filed in the Investigator's Trial File.

10.3 Other Reviewing Bodies

Health authorities will receive the protocol, SAE reports, and the final study report as required by institutional and federal regulations. In particular, review by FDA will or has been sought. These authorities will have access to source data collected during the study.

10.4 Monitoring requirements

The Principal Investigator will have the responsibility to oversee clinical monitoring of the clinical trial and will do so in association with the Clinical Trials Unit of the Translational Research Programs at the University of Pennsylvania. Staff of this unit have access to source documents to verify data on the CRF. The following data will be recorded and verified: demographics, medical history, date of signing of informed consent form, date of enrollment, visit dates, termination date, and primary safety variables.

10.5 Case Report Forms

CRFs will be generated by the Principal Investigator and completed by the Investigators or their data management staff using legible ballpoint pen with black ink. The Investigator or Investigator's authorized staff will ensure that all

information has been accurately transcribed and that correct dates and initials or signatures are present. By signing the last page of the CRF, the Investigator ensures that the information has been collected correctly. The Principal Investigator will sign an affirmation statement verifying that the content of the patient's CRF is accurate. Corrections to data on the CRFs will be made by drawing a straight line through the incorrect data and by writing the correct data next to the crossed out data. The correction will be initialed and dated by the Investigator or the Investigator's authorized staff member. Corrections made after the date of the Investigator's signature must be resigned and dated by the Investigator.

10.6 Quality Assurance

The trial will be conducted in accordance with the protocol, applicable regulatory requirements, and ICH Good Clinical Practices (GCP). All processes regarding the design, conduct, monitoring, evaluation, and reporting of the clinical trial may be subject to quality assurance audits to verify the validity, accuracy, and completeness of data. The Investigator permits any authorized audits or inspections that will become necessary within the framework of quality assurance activities. Information gathered during audits/inspections is considered confidential and will not be disclosed. All audits and inspections are documented and appropriate records are maintained.

10.7 Data Safety Monitoring Board (DSMB)

A data safety monitoring board (DSMB) will be created to ensure the safety of participants. The DSMB will include at least two medical doctors, a statistician and an administrative member to record minutes of the meetings. The DSMB

will be convened to review all safety data in the event of any Grade 3 or Grade 4 toxicity. The DSMB shall assess whether the toxicity is treatment-related and make a recommendation regarding additional dosing for other patients and dose escalation. The DSMB will review all AEs on each dose level before dose escalation is permitted.

10.8 Cost of tests and evaluations

Patients or their third party payer will be responsible for the costs of tests and evaluations conducted for this study that are part of standard of care. The study drug will be provided free of charge. The costs for this treatment, and any possible complications, side effects, and/or consequences are expected to be similar to the costs of alternative treatments for this disease.

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